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Pseudoscorpion diversity and distribution in the West Indies: sequence data confirm single island endemism for some clades, but not others

Julia G. Cosgrove[1,4], **Ingi Agnarsson**[2], **Mark S. Harvey**[3] and **Greta J. Binford**[1]: [1]Lewis & Clark College, Department of Biology, 0615 SW Palatine Hill Rd., Portland, Oregon, 97219, USA. E-mail: juliacosgrove@g.harvard.edu; [2]University of Vermont, Department of Biology, 109 Carrigan Drive, Burlington, Vermont, 05405, USA; [3]Department of Terrestrial Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia; [4]Current address: Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford St, Cambridge, Massachusetts, 02138, USA

Abstract. The Caribbean Islands are a biodiversity hotspot harboring high levels of endemic biodiversity. In an effort to contribute to the characterization of invertebrate diversity in the region, we present an assessment of pseudoscorpion (Arachnida: Pseudoseorpiones) diversity and distribution with a focus on the superfamily Chthonioidea and the family Olpiidae. We used three markers (cytochrome *c* oxidase subunit I, histone H3 and 28S rRNA) to infer the first molecular phylogenies for each lineage and identified 32 putative new speeies in need of taxonomic assessment. These new records include the documentation of the genera *Pseudochthonius* Balzan, 1892 *Lagynochthonius* Beier, 1951, *Tyrannochthonius* Chamberlin, 1929 (Chthoniidae), *Antillolpium* Muchmore, 1991, *Novohorus* Hoff, 1945, and *Pachyolpium* Beier, 1931 (Olpiidae) on various islands. Chthonioid genera are strongly structured geographically, suggesting that many Caribbean species may be short-range endemics and excellent candidate systems for testing biogeographic hypotheses. The olpiid genus *Pachyolpium* is less geographically structured, which is consistent with the hypothesis that olpiids are better dispersers than chthonioids. This study aims to provide a foundation for taxonomic and biogeographic work on Caribbean pseudoscorpions, revealing a diversity that is far richer than is documented in the literature.

Keywords: Caribbean, Pseudoscorpiones, phylogeography, dispersal, new records

When E.O. Wilson coined the term nesiophilia – an inordinate fondness and hungering for islands (Wilson 2010) – he had his fellow biogeographers in mind, who recognize the unique opportunities for diversification that isolated biological systems provide. Of critical importance in understanding island processes are the evolution and maintenance of endemic species, which are typically also 'short-range endemic' (SRE) species (Harvey 2002). Paired together with MacArthur and Wilson's classic theory of island biogeography (1967) and modern phylogenetic methods, SRE lineages become powerful not only for predicting species richness given island area and isolation, but also for informing our hypotheses about when, where, and how a lineage first colonized an island, and shedding light on the geological and evolutionary processes that drive diversification.

Caribbean biogeography.—The Caribbean islands (also commonly referred to as the West Indies, Fig. 1) are a natural laboratory for studying evolutionary processes (Ricklefs & Bermingham 2008) due to their varying degrees of isolation from the mainland and the heterogeneity of their geological histories. Additionally, the West Indies region was identified as one of 25 biodiversity hotspots for conservation priorities as characterized by high levels of endemicity in plants and vertebrates and high rates of habitat loss (Myers et al. 2000), although Aide et al. (2013) found that reforestation also played an important role in shaping the Caribbean landscape between 2001–2010. The Greater Antilles (Cuba, Jamaica, Hispaniola [Haiti and Dominican Republic], and Puerto Rico) are the largest of the Caribbean islands, and include a combination of fragment and non-volcanic Darwinian islands, many of which have been historically connected to continental landmasses and/or each other (Iturralde-Vinent 2006; Ricklefs & Bermingham 2008). As is predicted by the species-area relationship posited by MacArthur & Wilson (1967), these islands harbor the majority of Caribbean biodiversity, and similarly, within this system species richness is often highest on Cuba, followed by Hispaniola, then Jamaica and Puerto Rico (e.g., Losos 1996; Crews & Gillespie 2010; Alonso et al. 2012).

The Lesser Antilles, which span from the northernmost US/British Virgin Islands south to Trinidad & Tobago, and also include the former Netherlands Antilles (Aruba, Bonaire, and Curaçao), are smaller and mostly younger, volcanic, Darwinian islands that have never been connected to other landmasses (with a few exceptions, such as Trinidad; Ricklefs & Bermingham 2008). Lastly, the Bahamas and the Turks and Caicos Islands, made up of around 700 'platform islands', have always been adjacent to North America and have been intermittently submerged throughout their history (Ricklefs & Bermingham 2008, and geological references therein).

While isolation and area have been identified as two of the main abiotic factors that shape biogeographic patterns (theory of island biogeography), an organism's life history, potential and realized niche, evolutionary age, and dispersal capability (Claramunt et al. 2012; Agnarsson et al. 2014) are also key factors in determining its potential for colonization and subsequent diversification on an island (as well as its status as an SRE) (Harvey 2002; Lomolino 2010). For example, at the two extremes, poorly dispersing lineages typically have smaller ranges and are more geographically structured than lineages that disperse easily, as they are more likely to have established themselves on an island via a single chance colonization event or through vicariance. Groups that are better dispersers are more likely to lack biogeographic fidelity, making it difficult to infer their true geographic history. Here we assess the

Figure 1.—The Caribbean Islands, or West Indies, with sampled localities marked by white dots.

Caribbean diversity of one of the lesser-known arachnid orders, Pseudoscorpiones, in order to contribute to the characterization of the group's overall distribution and to identify particular groups that may be useful for testing biogeographic hypotheses.

Pseudoscorpiones.—Pseudoscorpions are small, inconspicuous arachnids found in terrestrial habitats all over the world; most commonly in leaf litter, but also on tidal flats, in caves, and in the cracks of bark and rocks (Weygoldt 1969; Murienne et al. 2008; Harvey 2013). All pseudoscorpions are predatory, and species within the suborder Iocheirata use venom secreted from one or more of their chelal fingers (distal 'hand' of the pedipalp) for prey capture (Chamberlin 1931; Harvey 1992). These animals are generally considered to be poor dispersers, although some exhibit phoretic behavior (i.e., individuals hitch rides on larger animals), allowing them to disperse as far as their hosts (e.g., Poinar et al. 1998; Zeh et al. 2003). While few phylogenetic analyses have been performed to establish relationships between and within the 25 families (~3,500 species) (Harvey 2013), molecular and morphological data support the order Pseudoscorpiones as monophyletic (Shultz 2007; Murienne et al. 2008).

The oldest documented pseudoscorpion fossil is from the mid Devonian (~380 million years old), and many younger fossils placed in extant families, including specimens from Dominican amber have also been described (e.g., Schawaller et al. 1991; Judson 2012; Harvey 2013). The Caribbean fossils suggest that several pseudoscorpion families have been present in this region for at least the last ~20 million years (Judson 1998), during which time some of the Greater Antilles split apart from each other (Pindell & Barrett 1990). Currently there are 147 extant species of pseudoscorpions (47 genera, 17 families) described from the Caribbean region, 120 of which are endemic to the Caribbean islands and 93 of which are single island endemics (Table 1; Harvey 2013). Further sampling may find that not all are truly restricted to single islands, or may show an even finer scale of species boundaries than currently appreciated. The diversity of Caribbean pseudoscorpions, both extant and extinct, as well as a wide range of dispersal abilities makes these animals excellent candidates for biogeographic analysis in this region.

Although considerable work has been done on vertebrate diversification in the Caribbean, few studies have analyzed the patterns and timing of colonization by invertebrates, and researchers have not yet identified any overarching principles

Table 1.—Total number of previously described Caribbean pseudoscorpion genera and species (Harvey 2013). Focal lineages are in boldface text.

Family	Genera	Species
Chthoniidae	**8**	**26**
Lechytiidae	**1**	**4**
Tridenchthoniidae	1	5
Bochicidae	4	9
Ideoroncidae	2	3
Syarinidae	3	11
Garypidae	1	1
Garypinidae	2	2
Geogarypidae	1	1
Olpiidae	**10**	**24**
Cheiridiidae	4	5
Pseudochiridiidae	1	1
Sternophoridae	2	3
Atemnidae	4	6
Cheliferidae	4	7
Chernetidae	22	34
Withiidae	4	5
Total	74	147

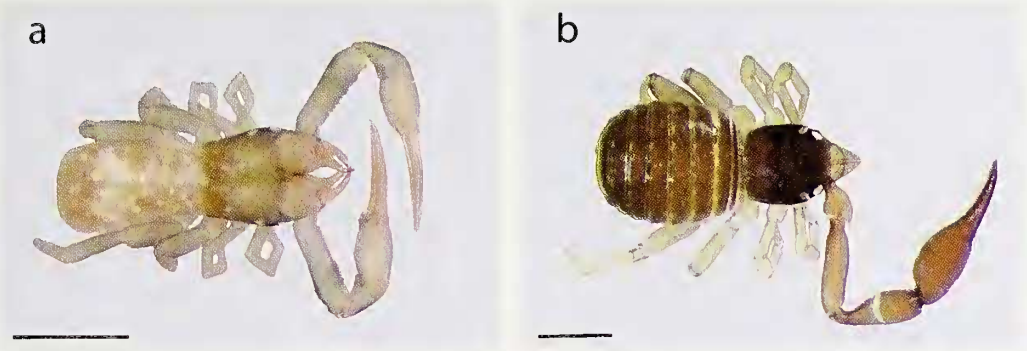

Figure 2.—Representative taxa: a, *Tyrannochthonius* sp.; b, *Pachyolpium cp033*. Scale bars represent 0.5 mm.

to explain the processes driving diversification in this biodiversity hotspot (*sensu* Myers 2000; Gillespie 2013). Nevertheless, an emerging pattern from the current project (CarBio, see islandbiogeography.org) indicates an important role of vicariant events and subsequent within-island radiation for various arachnid groups consisting of relatively poor dispersers (*Spintharus* Hentz, 1850: Dziki et al. 2015; *Phrynus* Lamarck, 1801: Esposito et al. 2015; *Micrathena* Sundevall, 1833: McHugh et al. 2014; *Loxosceles* Heineken & Lowe, 1832: Petersen et al. unpubl. data; *Deinopis* MacLeay, 1839: Chamberland et al. unpubl. data, and others). This study was undertaken to assess pseudoscorpion diversity in the Caribbean with a primary focus on two of the most diverse groups in the region: the superfamily Chthonioidea and the family Olpiidae (Fig. 2). We inferred the first molecular phylogenies for each clade in the Caribbean using three genes (cytochrome *c* oxidase subunit I (COI), 28S rRNA, and histone H3) and evaluated the phylogeographic structure of each lineage in order to identify patterns for future investigation.

METHODS

Taxon sampling and identification.—Specimens were collected into 95% ethanol by the CarBio field teams as part of an arachnid wide Caribbean inventory between 2010–2012. Pseudoscorpions were collected manually from trees, rocks, and sifted leaf litter, and extracted from litter using Berlese funnels. A variety of collecting methods decreases bias toward any particular taxonomic group or life stage; hand sorting tends to be biased towards larger and mature individuals, and Berlese funnels yield a more representative sample of juveniles and smaller individuals (Gabbutt 1970).

Using a stereomicroscope, specimens were first sorted to family using characters as described by Muchmore (1990) and Harvey (1992). Tissue samples (legs or a single pedipalp dissected from the body, depending on the size of the specimen) were taken for molecular work from 228 individuals belonging to Chthonioidea and Olpiidae, as these two groups were the most abundant in our collections (see Fig. 2 for images of the most highly represented genera in our samples). Specimens were then prepared for closer morphological examination using temporary slide mounts (as in Edward & Harvey 2008), and images of whole specimens, coxal spines (Chthonioidea), and chelal hands were taken through a compound (Chthonioidea) or light (Olpiidae) microscope using Automontage software. Each specimen was examined and diagnostic characters were compared to published descriptions of congeneric taxa in the Caribbean and adjacent regions. Individuals that matched these descriptions were identified to species, and individuals that did not match any published descriptions were identified to the genus level and further analyzed using the generated molecular data (see below). All specimens were returned to 95% ethanol after examination and stored at –20°C at Lewis & Clark College.

DNA extraction, amplification and sequencing.—DNA was extracted and purified from 96 specimens (of 228) using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) at Lewis & Clark College. DNA was extracted and purified from the remaining 132 specimens in the Smithsonian Laboratories of Analytical Biology (LAB) in Washington, DC using an Autogenprep965 for an automated phenol chloroform extraction (Smithsonian Institution 2013). All extractions were made from the four left legs and left pedipalp of chthonioids and the left pedipalp of olpiids.

Purified genomic DNA was used as a template to amplify cytochrome *c* oxidase subunit I (COI, ~1000 bp), histone H3 (H3, ~300 bp) and the large nuclear ribosomal subunit 28S rRNA (~1000 bp) (see Table 2 for primers and PCR conditions). COI and 28S rRNA have been useful for conducting phylogenetic analyses of pseudoscorpions at the genus level (Murienne et al. 2008), while histone H3 has been used to infer phylogenies and analyze evolutionary rates in other invertebrates (e.g. Colgan et al. 2000).

Table 2.—PCR conditions and target fragment length for each molecular marker.

Gene	Primers	MasterAmp™ buffer	Annealing temperature	Fragment length (bp)
COI	LCO1490/HCO2198	B	46.2°	~1000
28S	28spsF/28spsR	D	45°	~1000
H3	H3aR/H3nF	B	46.2°	~300

Table 3.—Taxa included in concatenated analyses.

	Species	Country/Island	Voucher	COI	28S	H3
Outgroups	*Feaella anderseni*	Australia	DNA 102369	EU559500.1	-	-
	Pseudogarypus bicornis	USA	DNA 102449	EU559501.1	EU559472.1	-
	Neopseudogarypus scutellatus	Australia	DNA 102431	EU559502.1	EU559456.1	-
	Afrosternophorus sp.	Australia	DNA 102437	EU559568.1	EU559461.1	-
	Lustrochernes sp.	Colombia	DNA 102430	EU559553.1	EU559455.1	-
	Ideoblothrus sp.	Colombia	DNA 102457	EU559562.1	EU559480.1	-
Chthonioidea	*Lagynochthonius* cp005	Puerto Rico	921A	KX263366	KX263326	KX263406
	Lagynochthonius cp006	Puerto Rico	782954	KX263365	KX263327	KX263407
	Lagynochthonius cp007	Puerto Rico	783084	KX263364	KX263328	KX263408
	Lagynochthonius proximus	Martinique	654A	KX263363	KX263325	KX263405
	Lechytia sini	Dominican Republic	782992	KX263367	KX263329	KX263409
	Pseudochthonius cp001	Dominican Republic	728A	KX263372	KX263333	KX263413
	Pseudochthonius cp001	Dominican Republic	782983	KX263375	KX263336	KX263416
	Pseudochthonius cp001	Dominican Republic	782996	KX263374	KX263338	KX263418
	Pseudochthonius cp001	Cuba	692A	KX263371	KX263332	KX263412
	Pseudochthonius cp001	Dominican Republic	917A	KX263373	KX263335	KX263415
	Pseudochthonius cp002	Mona	782995	KX263376	KX263337	KX263417
	Pseudochthonius cp003	Martinique	280A	KX263369	KX263330	KX263410
	Pseudochthonius cp003	Martinique	873A	KX263368	KX263334	KX263414
	Pseudochthonius cp004	Cuba	662A	KX263370	KX263331	KX263411
	Tyrannochthonius cp008	Cuba	986A	KX263394	KX263351	KX263431
	Tyrannochthonius cp009	Cuba	781A	KX263389	KX263343	KX263423
	Tyrannochthonius cp010	Cuba	931A	KX263380	KX263348	KX263428
	Tyrannochthonius cp011	Cuba	995A	KX263379	KX263352	KX263432
	Tyrannochthonius cp012	Cuba	675A	KX263390	KX263341	KX263421
	Tyrannochthonius cp013	Cuba	835A	KX263388	KX263346	KX263426
	Tyrannochthonius cp014	Dominican Republic	924A	KX263386	KX263347	KX263427
	Tyrannochthonius cp015	Dominican Republic	782997	KX263387	KX263355	KX263436
	Tyrannochthonius cp016	Cuba	805A	KX263385	KX263344	KX263424
	Tyrannochthonius cp017	Cuba	747A	KX263381	KX263342	KX263422
	Tyrannochthonius cp018	Cuba	942A	KX263382	KX263350	KX263430
	Tyrannochthonius cp019	Cuba	826A	KX263383	KX263345	KX263425
	Tyrannochthonius cp020	Cuba	655A	KX263384	KX263340	KX263420
	Tyrannochthonius cp021	Cuba	937A	KX263378	KX263349	KX263429
	Tyrannochthonius cp022	Mona	782976	KX263391	—	KX263435
	Tyrannochthonius cp023	Puerto Rico	782960	KX263393	KX263354	KX263434
	Tyrannochthonius cp024	Puerto Rico	782958	KX263392	KX263353	KX263433
	Tyrannochthonius insulae	Puerto Rico	782966	KX263377	KX263339	KX263419
Olpiidae	*Antillolpium* cp026	Dominican Republic	783047	KX263396	KX263357	KX263438
	Antillolpium cp027	Cuba	772A	KX263395	KX263356	KX263437
	Aphelolpium cp028	Puerto Rico	783054	KX263397	—	KX263439
	Apolpium parvum	Trinidad	DNA103134	EU559541.1	EU559489.1	—
	Pachyolpium cp029	Puerto Rico	783044	KX263402	—	KX263444
	Pachyolpium cp030	Puerto Rico	783059	KX263404	—	KX263446
	Pachyolpium cp031	Puerto Rico	783012	KX263400	KX263360	KX263442
	Pachyolpium cp032	Dominican Republic	783040	KX263401	KX263361	KX263443
	Pachyolpium cp033	Cuba	965A	KX263399	KX263359	KX263441
	Pachyolpium cp033	Cuba	866A	KX263398	KX263358	KX263440
	Pachyolpium cp033	Dominican Republic	783048	KX263403	KX263362	KX263445
	Pachyolpium sp.	Trinidad	DNA103132	EU559542.1	EU559488.1	—

For gDNA that was purified at LAB, COI was amplified and sequenced at the Smithsonian using LAB protocols (S.I. 2013). All other PCR amplifications were run at Lewis & Clark College. PCR products were validated using agarose gel electrophoresis (1% agarose), and successfully amplified reactions were cleaned up for sequencing with EXOSAP (0.5 μL/5 μL PCR product, 45 min incubation at 37°C and 15 min deactivation at 80°C). Final PCR products were Sanger-sequenced in both directions either at the University of Arizona Genomic Analysis and Technology Core, or at the LAB. Sequences for two chthonioid specimens and two olpiid specimens from Trinidad were obtained from GenBank (for vouchers see Table 3) and included in the analyses.

Sequence editing.—Sequences were assembled using SEQUENCHER 4.7 (Gene Codes Corp.), and contigs were aligned using MAFFT version 7 (Katoh 2013). MAFFT

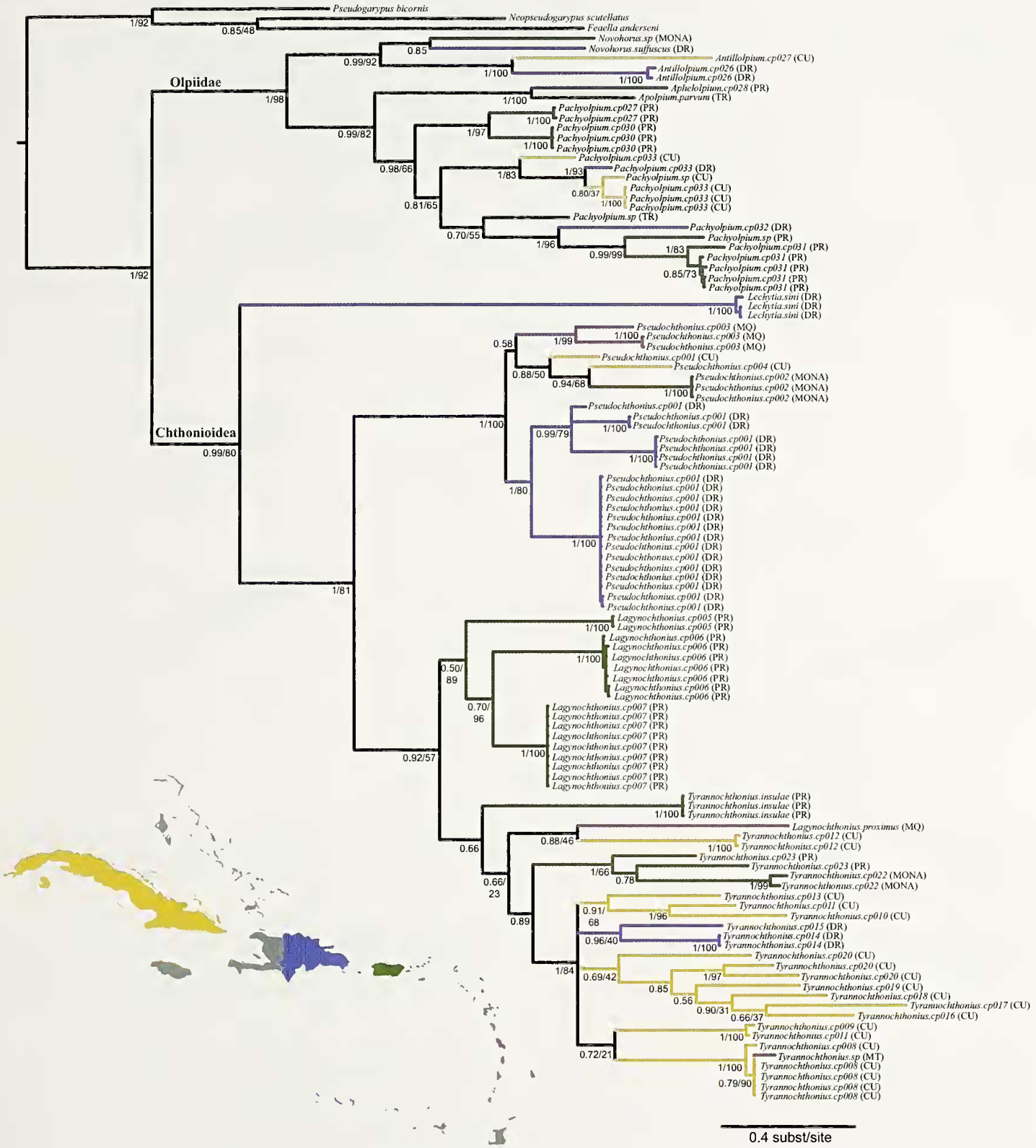

Figure 3.—Bayesian analysis of COI data from all sampled specimens supports Chthonioidea, Olpiidae, and most genera within these groups as monophyletic (all except *Lagynochthonius* + *Tyrannochthonius* which are recovered together in one clade). Posterior probabilities and bootstrap values are printed at each node for those nodes recovered by both MrBayes and RAxML (posterior probability/bootstrap value), and nodes recovered only in the Bayesian analysis are labeled with a single posterior probability value. Branches are colored by island and correspond to the colors on the map insert. CU = Cuba; DR = Dominican Republic; MQ = Martinique; MT = Montserrat; PR = Puerto Rico; TR = Trinidad.

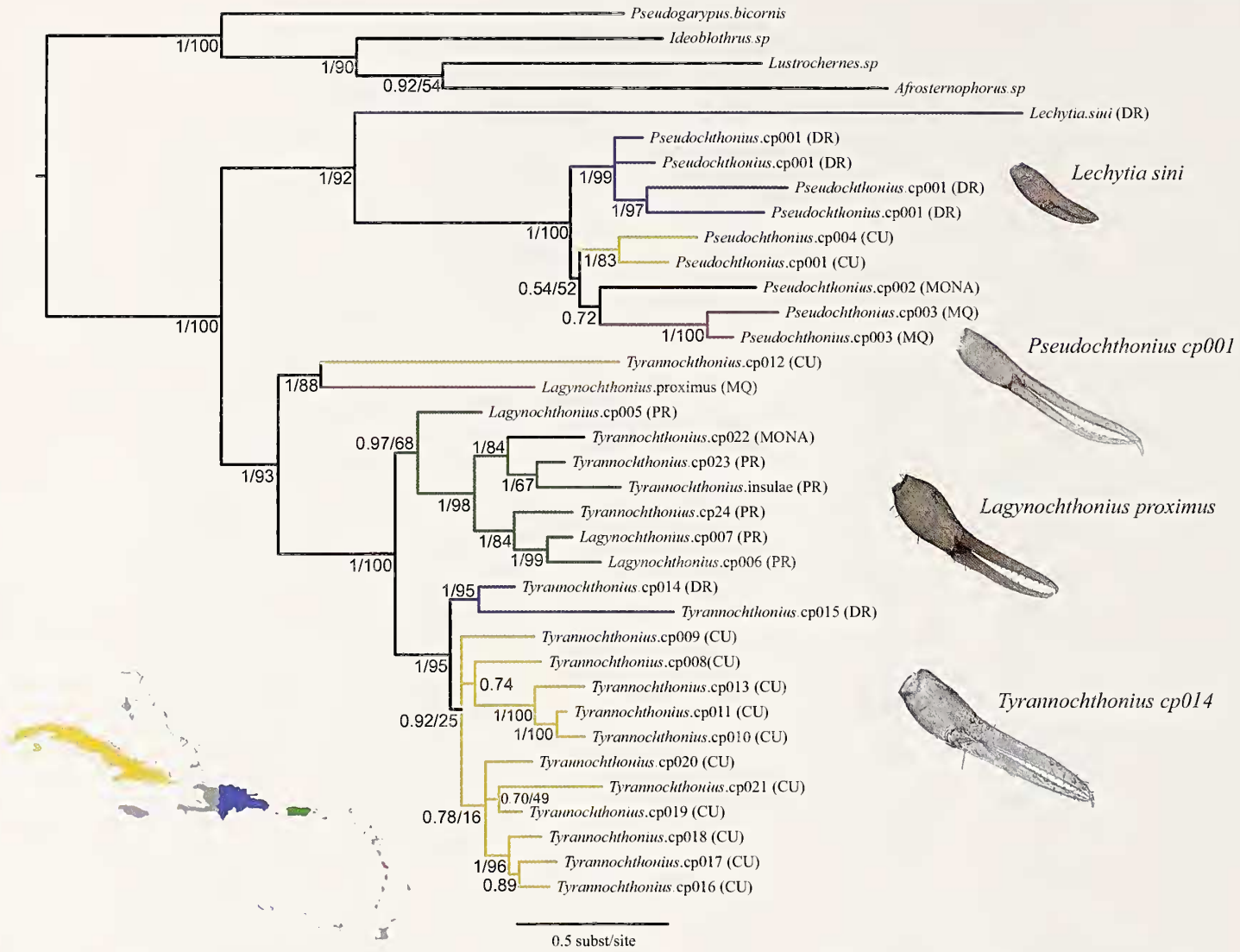

Figure 4.—Bayesian majority rule consensus tree of the concatenated matrix including COI, 28S, and H3 sequence data for Chthonioidea species and putative species. Posterior probabilities and bootstrap values are printed at each node for those nodes recovered by both MrBayes and RAxML (posterior probability/bootstrap value), and nodes recovered only in the Bayesian analysis are labeled with a single posterior probability value. Branches are colored by island and correspond to the colors on the map insert. CU = Cuba; DR = Dominican Republic; MQ = Martinique; PR = Puerto Rico.

settings changed from the default included: direction of nucleotide sequences [adjust direction according to the first sequence]; parameters, scoring matrix for nucleotide sequences [1PAM/k = 20]; align unrelated segments, too? [leave gappy regions]; unalignlevel [0.0]. Conserved blocks were selected using the less-stringent selection options in Gblocks version 0.91b (Castresana 2000), and the resulting alignments were used for all further analyses. Raw *p*-distances were calculated in Geneious v.8.1.7 (Kearse et al. 2012). All individuals for which COI successfully amplified were included in the complete COI analysis (n = 105), and individuals with sequence data from at least two genes were included in the final concatenated matrices (n = 42). Clades containing multiple individuals with identical COI haplotypes were pruned to include only one terminal in the concatenated datasets. MESQUITE (Maddison & Maddison 2011) was used to create concatenated matrices with all three genes for Chthonioidea and Olpiidae, and PartitionFinder v1.1.1 (Lanfear et al. 2012) was used to identify the best partitioning schemes for the concatenated analyses, defining seven possible partitions: 28S and positions one, two, and three for COI and H3, respectively.

Phylogenetic analyses.—RAxML version 8.2.3 (Stamatakis 2014) was used to run maximum likelihood (ML) analyses on an all-inclusive COI dataset through the CIPRES Science Gateway (Miller et al. 2010), as well as run ML analyses on the other individual genes and concatenated datasets for both Chthonioidea and Olpiidae (i.e., seven ML analyses in total; see Results, below). All ML analyses used the GTRGAMMA model with rapid bootstrapping (1000), specifying the random seed 555, and specifying the best partitioning scheme as identified by PartitionFinder (*raxmlHPC-HYBRID -T 4 -f a -n [alignment.file] -s [infile.txt] -N 1000 -p 555 –q [partition.file.txt] -m GTRGAMMA -x 555*).

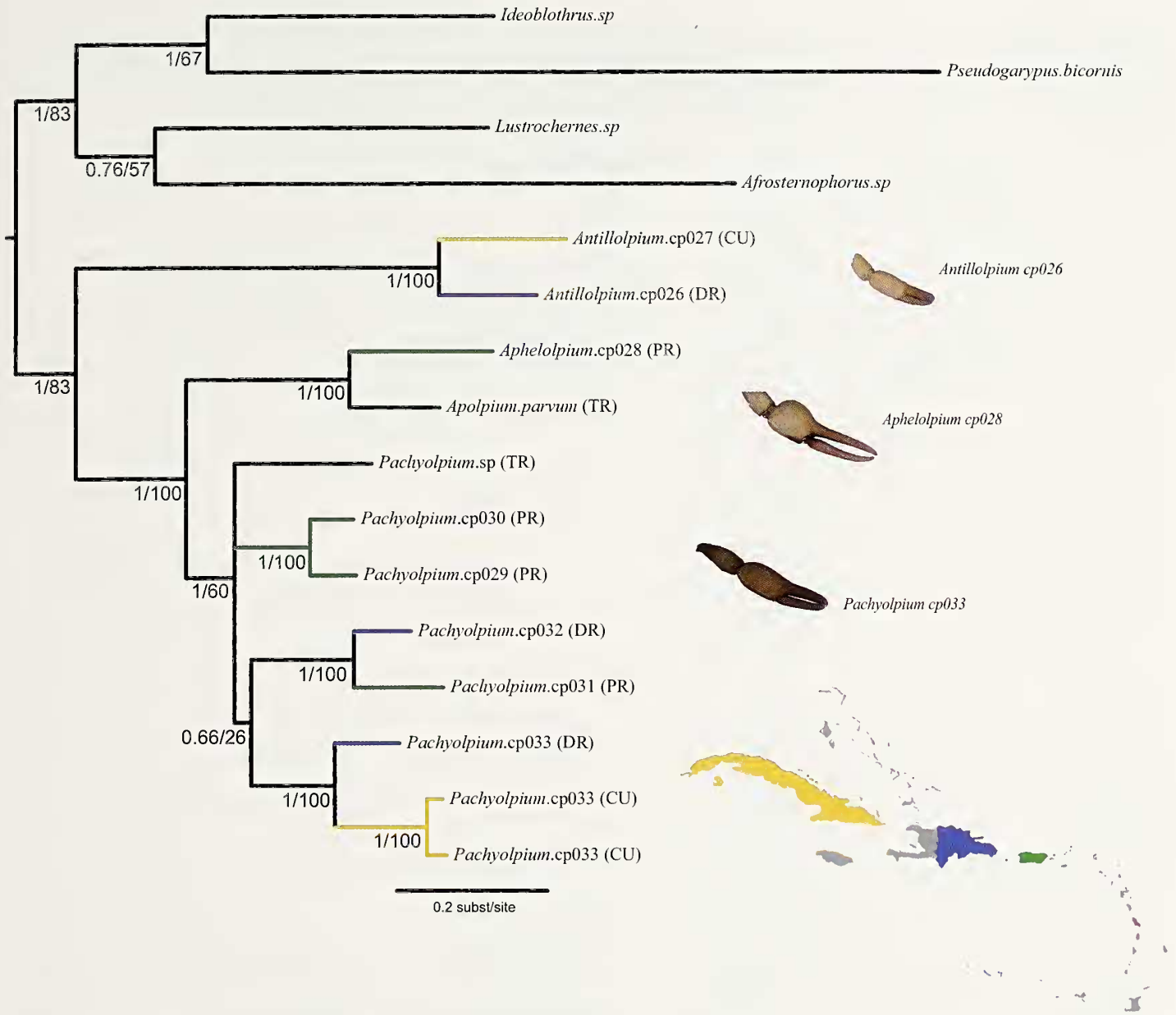

Figure 5.—Bayesian majority rule consensus tree of the concatenated matrix including COI, 28S, and H3 sequence data for Olpiidae species and putative species. Posterior probabilities and bootstrap values are printed at each node for those nodes recovered by both MrBayes and RAxML (posterior probability/bootstrap value), and nodes recovered only in the Bayesian analysis are labeled with a single posterior probability value. Branches are colored by island and correspond to the colors on the map insert. CU = Cuba; DR = Dominican Republic; MQ = Martinique; PR = Puerto Rico; TR = Trinidad.

Bayesian analyses were also run for the same seven datasets through the CIPRES Science Gateway (i.e., an all-inclusive COI dataset, as well as each individual gene matrix and the concatenated data sets for both Chthonioidea and Olpiidae) using MrBayes 3.2.1 (Huelsenbeck & Ronquist 2001). Evolutionary models selected using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) in jModelTest 2.1.7 v 20141120 (Darriba et al. 2012) were applied to the individual gene Bayesian analyses, and models selected using PartitionFinder were applied to each partition in the concatenated datasets. MrBayes was used to run two Markov chain Monte Carlo (MCMC) analyses of four chains each for 10 million generations (mcmc ngen = 10000000 nchains = 4 nruns = 2 temp = 0.1 samplefreq = 1000). The two independent MCMC runs were considered converged if the average standard deviation of split frequencies was ≤ 0.01, chain stationarity and mixing was confirmed in Tracer v1.6 (Rambaut & Drummond 2007), and a strong correlation between split frequencies in each run was confirmed using the AWTY online "compare" tool (Nylander et al. 2008). After discarding burn-in replicates, the remaining sampled trees were used to build a 50% majority-rule consensus tree, where the frequency of the nodes was represented by clade posterior probabilities.

The all-inclusive COI dataset with both chthonioids and olpiids was rooted with three pseudoscorpions belonging to

Table 4.—Distribution of Chthonioidea and Olpiidae in the Caribbean (Harvey 2013). First time records presented in this study are in boldface text.

	Genus	*Species*	Caribbean Distribution
Chthonioidea	*Aphrastochthonius*	*cubanus*	Cuba
	Caribchthonius	*butleri*	U.S. Virgin Islands
	Chthonius	*tetrachelatus*	Cuba
	Lagynochthonius	*callidus*	Jamaica
		cavicola	Jamaica
		inuoxius	Jamaica
		proximus	Dominican Republic, Jamaica
		typhlus	Jamaica
		dominicanus	Dominican Republic
			Martinique, Puerto Rico
	Paraliochthonius	*carpenteri*	The Bahamas
		insulae	Jamaica
		puertoricensis	Puerto Rico
	Pseudochthonius	*arubensis*	Aruba
		clarus	Jamaica
		doctus	Jamaica
		heterodentatus	Trinidad and Tobago
		insularis	St Vincent and the Grenadines
		inundanus	Jamaica
		thibaudi	Guadeloupe
			Isla Mona, Martinique, Cuba, Dominican Republic
	Tyrannochthonius	*bahamensis*	The Bahamas
		curazavius	Curaçao
		guadeloupensis	Guadeloupe
		hoffi	Jamaica
		inititatus	Dominican Republic, Jamaica
		insulae	Trinidad and Tobago
		ovatus	Martinique
			Cuba, Puerto Rico, Isla Mona
	Lechytia	*chthoniiformis*	Jamaica
		delamarei	Guadeloupe
		martiniquensis	Martinique
		trinitatis	Dominican Republic, Trinidad and Tobago
	Tridenchthonius	*cubanus*	Cuba, Jamaica
		dunaldi	Trinidad and Tobago
		gratus	Jamaica
		mexicanus	Trinidad and Tobago
		trinidadensis	Trinidad and Tobago
Olpiidae	*Aphelolpium*	*brachytarsus*	Aruba
		longidigitatum	Cayman Islands, Puerto Rico, U.S. Virgin Islands, Venezuela
		scitulum	Jamaica, Aruba, Bonaire, Curaçao
		thibaudi	Guadeloupe, Martinique
	Apolpium	*parvum*	Trinidad and Tobago
	Planctolpium	*arboreum*	Dominican Republic, Jamaica, Mexico
	Antillolpium	*cubanum*	Cuba
		hummelincki	Cayman Islands
			Dominican Republic
	Hoffhorus	*cinereus*	Trinidad and Tobago
	Leptolpium	*prospaeum*	Aruba, Bonaire, Curaçao
	Neopachyolpium	*longum*	Trinidad and Tobago
	Novohorus	*incertus*	Anguilla, St Martin, Puerto Rico, U.K. Virgin Islands, U.S. Virgin Islands,
		suffuscus	Jamaica, Mona, Puerto Rico
			Dominican Republic
	Olpiolum	*amphum*	Jamaica
		aureum	Mona, Puerto Rico
		confundens	Puerto Rico
		puertoricense	Puerto Rico
	Pachyolpium	*arubense*	Aruba, Bonaire, Curaçao, Klein Curaçao
		brevifemoratum	U.K. Virgin Islands
		brevipes	Martinique, St Vincent and the Grenadines
		confusum	St Eustatius

Table 4.—Continued.

Genus	*Species*	Caribbean Distribution
	furculiferum	Cayman Islands, St Vincent and the Grenadines, U.S. Virgin Islands, Venezuela
	isolatum	Jamaica, Panama
	medium	Dominican Republic, Jamaica, Puerto Rico, Florida
		Cuba

the superfamily Feaelloidea: *Feaella anderseni* Harvey, 1989, *Pseudogarypus bicornis* (Banks, 1895) and *Neopseudogarypus scutellatus* (Morris, 1948) (see Table 3 for GenBank accession numbers). The Feaelloidea are a basal group within Pseudoscorpiones consistently recovered as monophyletic (Harvey 1992; Murienne et al. 2008). The Chthonioidea and Olpiidae datasets (each with a concatenated matrix and two individual gene matrices for 28S and H3) were rooted with four pseudoscorpions belonging to four different superfamilies: *P. bicornis* (Feaelloidea), *Afrosternophorus* sp. (Sternophoroidea), *Lustrochernes* sp. (Cheliferoidea), and *Ideoblothrus* sp. (Neobisioidea).

Identifying putative species.—We used the Bayesian implementation of the Poisson tree processes model (bPTP) (http://www.exelixis-lab.org/, default parameters) to estimate the number of distinct species in our dataset including identical sequences that were removed for concatenation (Zhang et al. 2013). As this method tends to overestimate species richness when the numbers of individuals per haplotype are uneven (Zhang et al. 2013), and this was true for our dataset, we chose to use raw COI distances to identify putative species. Terminal taxa in the concatenated analyses that exhibited more than 10% divergence (uncorrected *p*-distances, COI) were defined as putative species and given a unique five-digit name beginning with *cp* (Caribbean pseudoscorpion), followed by three integers (001–033) assigned in order of morphological examination. We recognize that >10% COI divergence is neither a strict nor all-encompassing species-delimiting threshold, as Young & Hebert (2015) found that the average COI BIN (species proxy) distance within pseudoscorpion families is 0.190 (Demetras 2010), and up to 13.8% divergence has been observed between populations of the Neotropical pseudoscorpion species *Cordylochernes scorpioides* Linnaeus, 1758 (Wilcox et al. 1997), and up to 20% divergence between conspecifics of other arachnid lineages (Boyer et al. 2007; Fernández & Giribet 2014; Esposito et al. 2015). However, our main goal was not to define species within this subsample of Caribbean pseudoscorpions, but to assess the distribution of our focal lineages.

RESULTS

After alignment and selection of conserved blocks our final matrices were structured as follows: COI Chthonioidea + Olpiidae (taxa = 110, sites = 647 [95% of original alignment]); 28S Chthonioidea (taxa = 35, sites = 926 [81% of original alignment]); 28S Olpiidae (taxa = 13, sites = 1051 [90% of original alignment]); H3 Chthonioidea (taxa = 32, sites = 287 [100% of original alignment]); H3 Olpiidae (taxa = 10, sites = 375 [98% of original alignment]); concatenation Chthonioidea (taxa = 36, sites = 1915); concatenation Olpiidae (taxa = 16, sites = 2079).

The best fitting evolutionary models identified for our individual gene data sets were as follow: Chthonioidea + Olpiidae COI (GTR + G + I); Chthonioidea & Olpiidae 28S (GTR + G + I); Chthonioidea H3 (SYM + G); Olpiidae H3 (K80 + G). The best partitioning scheme identified for the concatenated Chthonioidea dataset included three subsets: [(COI_codon1, COI_codon3), (COI_codon2, H3_codon1, H3_codon2), (28S, H3_codon3)], for which the best-fit evolutionary models were identified as GTR+I+G, SYM+I+G and GTR+I+G, respectively. The best partitioning scheme identified for the concatenated Olpiidae dataset included seven subsets, one for each codon position in COI and H3 and one for 28S. The best-fit evolutionary models identified for these subsets were: COI_codon1 (GTR+I+G); COI_codon2 (F81+G); COI_codon3 (HKY+I+G); 28S (GTR+I+G); H3_codon1 (SYM); H3_codon2 (JC); and H3_codon3 (K80+I).

Phylogenetic analyses.—Convergence between runs was supported for each of our Bayesian analyses as defined by an average standard deviation of split frequencies ≤ 0.01, stationarity and mixing visualized in Tracer v1.6, and a strong correlation observed between run split frequencies using AWTY. Stationarity was achieved by one million generations (1000 sampled trees) in each of our analyses, so we used burn-in values of 1000 (10%) to summarize statistics in MrBayes.

Gene tree topologies differed slightly between the two phylogenetic methods. Both ML and Bayesian inference using our COI dataset recovered Chthonioidea and Olpiidae as monophyletic (posterior probability = 1, bootstrap value = 92), however our ML analysis only recovered six of nine genera as monophyletic while Bayesian inference recovered seven of nine. Both methods yielded identical topologies for our 28S matrices and the Chthonioidea H3 matrix, but ML analysis of our olpiid H3 matrix recovered *Apheloipinm* Hoff, 1964 nested within the *Pachyolpium* Beier, 1931 clade while Bayesian inference recovered *Apheloipinm* as sister to the *Pachyolpium* clade. Between methods, the topologies inferred from our concatenated datasets were identical for Olpiidae and nearly identical for Chthonioidea. Trees shown here are Bayesian majority rule consensus trees (Figs. 3–5). The three differences observed within Chthonioidea occurred at the putative species level within poorly resolved clades (see Figs. 4, 5).

Chthonioidea.—Our concatenated molecular phylogenetic analysis included 32 genetically distinct terminal taxa in the superfamily Chthonioidea, representing four genera: *Lechytia* Balzan, 1892, *Pseudochthonius* Balzan, 1892, *Tyrannochthonius* Chamberlin, 1929 and *Lagynochthonius* Beier, 1951 (Fig. 4).

Table 5.—Raw COI p-distances between putative *Pseudochthonius* species (*P.*), putative *Tyrannochthonius* and *Lagynochthonius* (*T.* and *L.*), and putative Olpiidae species.

	P. cp003 (MQ)	*P. cp003* (MQ)	*P. cp004* (CU)	*P. cp001* (CU)	*P. cp001* (DR)	*P. cp001* (DR)	*P. cp001* (DR)	*P. cp001* (DR)
P. cp003 (MQ)	-							
P. cp003 (MQ)	0.094	-						
P. cp004 (CU)	0.136	0.116	-					
P. cp001 (CU)	0.114	0.118	0.101	-				
P. cp001 (DR)	0.132	0.123	0.114	0.092	-			
P. cp001 (DR)	0.116	0.127	0.116	0.107	0.118	-		
P. cp001 (DR)	0.112	0.114	0.119	0.09	0.092	0.078	-	
P. cp001 (DR)	0.112	0.121	0.128	0.09	0.103	0.099	0.058	-
P. cp002 (MONA)	0.134	0.152	0.121	0.11	0.137	0.128	0.132	0.141

	T. cp021 (CU)	*T. cp011* (CU)	*T. cp010* (CU)	*T. cp017* (CU)	*T. cp018* (CU)	*T. cp019* (CU)	*T. cp020* (CU)	*T. cp016* (CU)
T. cp021 (CU)	-							
T. cp011 (CU)	0.172	-						
T. cp010 (CU)	0.195	0.110	-					
T. cp017 (CU)	0.172	0.174	0.175	-				
T. cp018 (CU)	0.157	0.161	0.179	0.137	-			
T. cp019 (CU)	0.154	0.146	0.179	0.168	0.139	-		
T. cp020 (CU)	0.159	0.146	0.174	0.139	0.157	0.134	-	
T. cp016 (CU)	0.161	0.166	0.179	0.145	0.136	0.152	0.152	-
T. cp015 (DR)	0.163	0.161	0.192	0.166	0.175	0.179	0.156	0.163
T. cp014 (DR)	0.165	0.157	0.165	0.175	0.159	0.177	0.157	0.163
T. cp013 (CU)	0.17	0.165	0.179	0.186	0.163	0.172	0.154	0.154
T. cp009 (CU)	0.177	0.166	0.204	0.163	0.170	0.165	0.163	0.179
T. cp022 (MONA)	0.163	0.165	0.179	0.175	0.172	0.161	0.152	0.179
T. cp024 (PR)	0.17	0.163	0.192	0.175	0.165	0.177	0.165	0.179
T. cp023 (PR)	0.165	0.179	0.186	0.184	0.177	0.177	0.181	0.177
T. cp012 (CU)	0.19	0.192	0.195	0.163	0.168	0.184	0.166	0.188
T. cp008 (CU)	0.174	0.172	0.165	0.172	0.172	0.165	0.159	0.184
L. cp005 (PR)	0.186	0.190	0.193	0.195	0.199	0.193	0.166	0.199
L. cp007 (PR)	0.166	0.174	0.197	0.179	0.175	0.177	0.156	0.172
L. cp006 (PR)	0.179	0.197	0.206	0.201	0.204	0.188	0.188	0.157
L. proximus (MQ)	0.201	0.186	0.208	0.208	0.186	0.213	0.195	0.204
T. insulae (PR)	0.197	0.206	0.217	0.222	0.206	0.212	0.172	0.212

	Antillolpium cp026 (DR)	*Antillolpium cp027* (CU)	*Aphelolpium cp028* (PR)	*Apolpium parvum* (TR)	*Pachyolpium cp029* (PR)	*Pachyolpium cp030* (PR)	*Pachyolpium cp033* (CU)	*Pachyolpium cp033* (CU)
Antillolpium cp026 (DR)	-							
Antillolpium cp027 (CU)	0.203	-						
Aphelolpium cp028 (PR)	0.256	0.271	-					
Apolpium parvum (TR)	0.251	0.298	0.178	-				
Pachyolpium cp029 (PR)	0.241	0.256	0.216	0.218	-			
Pachyolpium cp030 (PR)	0.246	0.238	0.218	0.213	0.113	-		
Pachyolpium cp033 (CU)	0.223	0.258	0.203	0.208	0.160	0.170	-	
Pachyolpium cp033 (CU)	0.253	0.271	0.231	0.218	0.178	0.175	0.108	-
Pachyolpium cp033(DR)	0.241	0.263	0.223	0.221	0.163	0.165	0.090	0.065
Pachyolpium sp (TR)	0.258	0.271	0.231	0.211	0.173	0.170	0.175	0.168
Pachyolpium cp031 (PR)	0.283	0.296	0.253	0.258	0.228	0.221	0.198	0.203
Pachyolpium cp032 (DR)	0.248	0.296	0.251	0.231	0.201	0.203	0.198	0.213
Novohorus cp025 (MONA)	0.193	0.216	0.236	0.226	0.211	0.216	0.206	0.216
Novohorus suffuscus (DR)	0.208	0.233	0.208	0.251	0.213	0.213	0.193	0.195

Table 5.—Extended.

P. cp002 (MONA)	*T. cp015* (DR)	*T. cp014* (DR)	*T. cp013* (CU)	*T. cp009* (CU)	*T. cp022* (MONA)	*T. cp024* (PR)	*T. cp023* (PR)	*T. cp012* (CU)	*T. cp008* (CU)	*L. cp005* (PR)	*L. cp007* (PR)	*L. cp006* (PR)	*L. proximus* (MQ)	*T. insulae* (PR)	*Pachyolpium cp033* (DR)	*Pachyolpium* sp (TR)	*Pachyolpium cp031* (PR)	*Pachyolpium cp032* (DR)	*Novohorus cp025* (MONA)	*Novohorus suffuscus* (DR)
-																				
0.146	-																			
0.154	0.159	-																		
0.165	0.174	0.170	-																	
0.168	0.190	0.168	0.184	-																
0.168	0.166	0.192	0.183	0.134	-															
0.159	0.193	0.199	0.208	0.136	0.141	-														
0.192	0.192	0.184	0.188	0.159	0.181	0.150	-													
0.163	0.175	0.174	0.163	0.165	0.159	0.161	0.148	-												
0.184	0.203	0.193	0.201	0.166	0.177	0.201	0.212	0.193	-											
0.168	0.181	0.188	0.179	0.165	0.163	0.157	0.170	0.168	0.141	-										
0.183	0.186	0.190	0.197	0.190	0.188	0.197	0.197	0.206	0.172	0.125	-									
0.206	0.193	0.208	0.230	0.192	0.188	0.199	0.166	0.192	0.206	0.195	0.213	-								
0.188	0.208	0.213	0.201	0.172	0.201	0.186	0.186	0.174	0.184	0.170	0.192	0.199	-							
															-					
															0.175	-				
															0.188	0.195	-			
															0.193	0.190	0.208	-		
															0.211	0.233	0.273	0.246	-	
															0.198	0.216	0.256	0.253	0.148	-

Three of these taxa were described species that had previously been documented in the Caribbean region: *Lechytia sini* Muchmore, 1975, *Lagynochthonius proximus* (Hoff, 1959) and *Tyrannochthonius insulae* (Hoff, 1946), while the remaining taxa represent 24 putative new species. The bPTP model estimated 38 distinct species from the same dataset. These include the first *Pseudochthonius* species recorded from Isla Mona, Martinique, Cuba, and the Dominican Republic (except for extinct *Pseudochthonius squamosus* Schawaller, 1980 found in Dominican Amber), the first *Tyrannochthonius* species from Isla Mona and Cuba, and the first *Lagynochthonius* species from Puerto Rico and Martinique (Table 4).

The smallest raw COI *p*-distance between putative *Pseudochthonius* species was 0.101 and between *Tyrannochthonius* and *Lagynochthonius* species 0.110 (Table 5). Within the superfamily, *Tyrannochthonius* + *Lagynochthonius* formed a monophyletic group as did *Pseudochthonius* and *Lechytia* (Fig. 4). Within the *Pseudochthonius* clade, each individual island was monophyletic (Fig. 4), although relationships among islands were not resolved. Within the *Tyrannochthonius* + *Lagynochthonius* clade, individuals from Dominican Republic and Puerto Rico/Mona Island were monophyletic and individuals from Cuba were polyphyletic, due to a single rogue taxon (Fig. 4).

Olpiidae.—Our concatenated molecular phylogenetic analysis included 12 genetically distinct terminal taxa representing four genera: *Antillolpium* Muchmore, 1991, *Aphelolpium*, *Apolpium* Chamberlin, 1930, and *Pachyolpium*. One of these taxa was a previously described species: *Apolpium parvum* Hoff, 1945 from Trinidad (sequence data from Murienne et al. 2008), another was an undescribed species also from Trinidad: *Pachyolpium* sp. (sequence data from Murienne et al. 2008), and the remaining 10 taxa represent eight putative new species. The bPTP model estimated 15 distinct species from the same dataset. The Olpiidae specimens include the first records of *Antillolpium* from the Dominican Republic and the first *Pachyolpium* species from Cuba (Table 4). Additionally, our samples also contained the first records of the olpiid genus *Novohorus* Hoff, 1945 from the Dominican Republic, however these specimens did not yield sufficient molecular data to be included in the concatenated dataset. The smallest raw COI *p*-distance between putative *Pachyolpium* species was 0.113 (Table 5). The genus *Pachyolpium* formed a monophyletic group, and within this clade individual specimens from Cuba and Trinidad were monophyletic; individuals from Puerto Rico and Dominican Republic were polyphyletic (Fig. 5). The other olpiid genera are not discussed due to small sample sizes.

DISCUSSION

Our initial assessment of pseudoscorpion diversity in the Caribbean has focused on only a fraction of the order: nine of 47 known genera, and only seven of the 41 known species within those genera (Harvey 2013). We found 32 genetically distinct taxa that are also morphologically distinct from currently described Caribbean species and warrant closer taxonomic assessment (Figs. 3–5). We also documented first time island records of six genera: *Pseudochthonius, Lagynochthonius, Tyrannochthonius, Antillolpium, Novohorus* and *Pachyolpium* (Table 4). While geographic coverage is not dense within any genus, this sampling allows for a preliminary assessment of island-level monophyly for a few genera.

Chthonioidea.—Relationships among Caribbean chthonioid genera in our analyses are consistent with previous systematic hypotheses (Murienne et al. 2008). In an order-wide molecular phylogeny, *Pseudochthonius* and *Lechytia* formed part of a larger clade that also included *Anaulacodithella* Beier, 1944 and *Sathrochthonius* Chamberlin, 1962, both of which are temperate Gondwanan groups (Murienne et al. 2008). The genera *Tyrannochthonius* and *Lagynochthonius* were also found to be closely related (Murienne et al. 2008). The *Lechytia* + *Pseudochthonius* clade inferred in the current study contains *Lechytia sini* from the Dominican Republic, which is sister to four putative *Pseudochthonius* species (Fig. 4). While we only have at most four terminal taxa on any particular island, the *Pseudochthonius* putative species groups form island clades (Fig. 4). This geographic structure is consistent with low expected dispersal within the group, and suggests that the biogeographic history of *Pseudochthonius* species in the Caribbean may reflect geological events.

The *Tyrannochthonius* + *Lagynochthonius* clade is the most diverse in our analysis, with 22 species including 20 putative new species and raw *p*-distances ranging from 0.110 to 0.230 (Table 5). It is most likely that this group represents one or a few undescribed species complexes, as 10% divergence in COI exceeds typical, though arbitrary, species delimitation thresholds. This clade also contains two notable within-island radiations: six putative *Tyrannochthonius* + *Lagynochthonius* species on Puerto Rico, and 12 putative *Tyrannochthonius* species on Cuba (Fig. 4), where this genus has not been previously documented. Further morphological and molecular analyses will be necessary to determine the taxonomic status of these putative species, however after examination of diagnostic characters, we are confident that they do not fit any published species description.

Tyrannochthonius and *Lagynochthonius* species are nested in one clade with no clear genetic distinction, which is consistent with the historic paraphyly of these groups [*Lagynochthonius* was considered a subgenus of *Tyrannochthonius* until 1962 (Chamberlin 1962), and the taxonomic status of this group is still debated], and with the results of a study on Australian members of these genera (Harrison et al. 2014). Despite this paraphyly, the geographic structure of this group is still notable. This structure, as well as previous work showing that hypogean *Tyrannochthonius* and *Lagynochthonius* species in Western Australia are SREs (Edward & Harvey 2008; Harrison et al. 2014), calls for thorough biogeographical analysis of these groups in the Caribbean region.

Within our chthonioid dataset the total number of putative species is highest on Cuba (14), which is consistent with the species-area relationship discussed by MacArthur & Wilson (1967), however only four putative species were found on Hispaniola while six were found on Puerto Rico. This could be an artifact of Hispaniola only being represented by the Dominican Republic in our study. When our putative species are added to the currently described species lists for these three islands, diversity is consistent with species-area relationships (Cuba: 17; Hispaniola: 8; Puerto Rico: 9).

Olpiidae.—Our molecular phylogenetic analysis of Caribbean olpiids is consistent with current taxonomic rankings, as

each currently described genus forms a monophyletic group. The subfamily Hesperolpiinae is represented by two genera, *Aphelolpium* and *Apolpium*, which form a clade nested within the rest of the olpiids belonging to the subfamily Olpiinae: *Antillolpium* and *Pachyolpium* (Fig. 5). The relationship between these two subfamilies remains unclear, and a thorough molecular and morphological analysis will be necessary to resolve the Olpiidae phylogeny.

Polyphyletic island groups within the genus *Pachyolpium* indicate multiple dispersal events (Fig. 5), although this genus is not strongly supported in our ML concatenated dataset (posterior probability = 1, bootstrap value = 60). In our COI analysis, *Pachyolpium* has higher bootstrap support (posterior probability = 0.98, bootstrap value = 66, Fig. 3), but more thorough sampling will be necessary to infer the true biogeographic history of olpiids in the Caribbean. Should further biogeographic analyses find patterns consistent with olpiids dispersing between islands more frequently than expected for a non-phoretic lineage and thus more frequently than chthonioids, we propose two hypotheses: (1) that olpiids are typically found in more xeric environments than chthonioids, and (2) may therefore be better suited to colonizing drier, coastal environments after an initial dispersal event (Wilson 1959; Judson 2003). Although the 'predation hypothesis' which states that phoresy in pseudoscorpions is a byproduct of predation (Vachon 1940, 1954; Muchmore 1971) was rejected by Zeh & Zeh (1992), it is possible that the pedipalp morphology of venomous pseudoscorpions (including Olpiidae) is more conducive to latching onto a larger, flying arthropod than that of the non-venomous pseudoscorpions (including Chthonioidea), which tend to have longer, more slender palpal fingers (see Figs. 2, 4, 5).

Within the olpiids, the number of putative species is highest on Puerto Rico (4), followed by Hispaniola (3), and Cuba (2). This trend is upheld when previously described olpiid species are also added to the list: Puerto Rico: 11; Hispaniola: 5; Cuba: 3. As the classic species-area relationship has been suggested to be driven primarily by *in situ* diversification (Losos & Parent 2010), the opposite pattern observed in olpiids is consistent with dispersal playing a dominant role in shaping their diversity in the Caribbean.

In conclusion, this study suggests that there is a great wealth of undocumented pseudoscorpion diversity in the Caribbean. A more thorough sampling and morphological assessment will elucidate how many new species and/or species complexes these genetically distinct taxa represent. Species of *Tyrannochthonius*, *Lagynochthonius* and *Pseudochthonius* form island specific clades, suggesting that they may be short-range endemics and thus highly informative to biogeographers and conservation biologists. Species of *Pachyolpium* form polyphyletic island groups, suggesting that they have likely dispersed between islands multiple times. More sampling within genera across the Caribbean and from adjacent continents will allow us to infer the directionality of dispersal and time-calibrate these phylogenies, empirically testing the biogeographical hypotheses inspired by the present data. There is a great need for integrated taxonomic research on these lineages in order to understand more deeply their diversity, distributions, evolutionary histories and taxonomy.

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